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THE SEAT OF SMELL IN THE CRAYFISH.

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The location of the sense of smell in the crustacea has been the subject of but few investigations. Putnam, Graber, Bateson, Dearborn, Spaulding and others have made observations on the reactions of various decapods to food at a distance from the body, although they were not primarily concerned in tracing the seat of the olfactory reactions. Herrick, working on the lobster, found that most parts of the body were sensitive to ammonia and clam juice. Experiments of Nagel [1] and Bethe [2] indicate that, in the decapods, the first antennæ have an important olfactory function, although both these investigators regard the olfactory sense organs as not exclusively located in these appendages. Nagel worked with *Pagurus*, *Astacus* and *Carcinus*. Removal of the antennules of *Pagurus* was followed by reactions to chemical stimuli, although these were not so pronounced as before the operation. *Astacus* in the presence of food was found to show lively movements of the antennules, followed by movements of the mouth parts and legs. *Carcinus* showed the same movements of the mouth parts and antennæ in the presence of food, but it was only when food came into actual contact with the mouth parts that efforts were made to secure it. Nagel's experiments led him to conclude that sight and touch play a greater part in locating food than the sense of smell.

Bethe found that if a piece of meat is thrown into a dish with several *Carcinus* there is no reaction at first. After several seconds the antennules begin to wave rapidly and the maxillipeds move slowly back and forth. The animals nearest the piece of meat

[1] Bibliotheca Zoologica, Bd. 18, 1894.

[2] *Archiv f. mik. Anat.*, Bd. 50, 1897.

give this reaction first. The farther the *Carcinus* is from the meat the later will begin the reactions. Soon after the animals begin to react they move about and usually in quite a direct line toward the meat. The nearer they approach the food the faster they go. That the eye plays little part is evidenced by the fact that blinded forms go toward the meat with just as much certainty. If a piece of meat is passed over the rocks at the bottom of the aquarium a *Carcinus* will follow this scent and often pick up every stone, which has been in contact with the meat, and pass it to the mouth, but as soon as the stones come in contact with the maxillipeds they are rejected.

If the antennules are removed *Carcinus* reacts to food but only when it is placed a short distance from the mouth parts. The latter are regarded by Bethe as also sensitive to chemical stimuli. In the amphipod *Amphithoe*[1] one of the present writers found that the antennules were important olfactory organs, but that there remained a certain but much diminished power to detect food at a distance from the mouth parts after the antennules, or even the antennules and the antennæ were removed. Other species of amphipods were found to make exploring movements of the antennules when in the presence of food.

Nagel's experiments with amphipods and isopods failed to elicit any positive response, although some species gave negative responses to disagreeable or irritating substances. The terrestrial isopods, *Oniscus*, *Porcellio* and *Armadillo* he found to be quite insensitive to strong substances like cedar oil and benzol. It is not difficult, however, to obtain very decided reactions from terrestrial isopods to the vapor of acetic acid and ammonia even after removal of the antennæ.

Many papers have appeared dealing with the organs of smell in crustacea but conclusions regarding their functions have been based more on morphological grounds than on the results of experiment. One of the most recent papers on the chemical sense of crustacea is that of Bell,[2] who worked on the reactions of the crayfish. Bell applied meat juice by means of a fine-pointed pipette, to various parts of the body. To render it evi-

[1] BIOLOGICAL BULLETIN, Vol. 2, p. 165, 1901.

[2] *Jour. Comp. Neur. and Psych.*, 16, 299, 1906.

dent when the substance came in contact with the animal, it was colored with eosin or carmine. It was found that stimulation of various parts of the body would elicit a response. The animal moved its mouth parts, became restless, moved the small chelipeds about and sooner or later attempted to go toward the source of stimulus. The responses varied somewhat according to the region to which the stimulus was applied, but there was a marked tendency, as we have also found, for the first reaction to manifest itself by a movement of the part most directly stimulated. The antennules, antennæ, mouth parts and tips of the large and small chelipeds were found to be organs of especial sensitiveness. We have repeated Bell's experiments with meat juice and verified in the main his results. Bell, however, did not test his conclusions by removal of certain organs or by operations on the nervous system. One is never quite sure that by using chemicals which diffuse through the water only the organ is stimulated to which the stimulus is applied. It was thought desirable, not only for this reason, but on account of determining more definitely the role of certain organs in chemoreception to observe the reactions of crayfish after the removal of the antennules and antennæ, and after the destruction of the brain or the division of the nerve cord.

The outer ramus of the antennules bears, in addition to the kinds of setæ found on the antennæ and other parts of the body, certain peculiar club-shaped organs which have generally been considered the end organs of the sense of smell. These organs are absent on the inner ramus, and on the antennæ as well as other parts of the body. Organs of a similar character are common in other crustacea, being in many cases more abundantly developed in the male. This circumstance, as well as the fact that removal of the antennules diminishes the response to olfactory stimuli, renders it quite probable that these club-shaped organs perform the function which has so often been assigned to them. In a series of experiments we have compared the reactions of crayfish with the outer ramus of the antennules removed with the reactions of normal animals and individuals otherwise operated on. The rami were removed several days before the animals were experimented with in order to eliminate any effect of shock

on the reactions. All of the specimens were kept for the same period without food, were placed in the same kind of dishes and treated in all respects as nearly as possible in the same way. Only individuals which had been resting quietly for some minutes were employed for experiment. If any reaction was produced by the movements of the observer the crayfish was not used for experiment until it had been left for a considerable time and became perfectly quiet. A piece of meat was very carefully introduced into the water with a long fine forceps and held a quarter of an inch above the rostrum. If one's movements are exceedingly slow it is possible to work around crayfish even when recently brought in from the field without exciting alarm. The number of seconds was noted before any of the feeding responses of the legs or mouth parts were set up. Sometimes such movements appear to arise spontaneously, and therefore only those cases were counted in which these movements eventuated in seizing and devouring the meat. In three sets of experiments performed at different times the time reactions of the crayfish were as follows:

EXPERIMENT I.

	Time of Reactions in Seconds.								Average.
Outer ramus removed		60	80	42	16	36	220	64	74
Inner ramus removed		80	34	64	74	30	6		48
Both rami removed	110	170	270	88	180	156	58	116	131
Normal animal	46	12	6	30	36	68	30	24	31

EXPERIMENT II.

Outer ramus removed	184	148	92	272	174
Inner ramus removed	36	20	24		27
Both rami removed	50	64	120	90	81
Normal animal	2	18	6		8.7

EXPERIMENT III.

Outer ramus removed	10	24	26	24	44	25.6
Inner ramus removed	10	18	24	42		23
Both rami removed	112	120	412	26		167.5
Normal animal	26	16	26			22.7

EXPERIMENT IV.

(Specimens with eyes blackened over.)

Outer ramus removed	72	116	180	120	36	94	30	110	144			100
Inner ramus removed	6	12	4	4	4	6	36	12	4	48	14	13.7
Both rami removed		42	112	242	270							141.5

EXPERIMENT V.

(Specimens with eyes blackened over.)

Outer ramus removed	74	68	214	56	168						118
Inner ramus removed	10	42	14	32	102	32	34	27	6	33	33.2

The experiments show in a striking enough way that removal of the outer branch of the antennules is followed by a much greater loss of sensitiveness to olfactory stimuli than is caused by removal of the inner ramus. But they also show that crayfish with the inner ramus removed react less promptly than do normal animals. We may conclude from these experiments that the part most sensitive to olfactory stimuli is the outer ramus of the antennules, but that the inner ramus is also to a certain extent sensitive to the same kind of stimuli. Specimens with both rami removed respond to meat juice or pieces of meat placed near the tip of the large second antennæ. It is scarcely possible that any chemical could diffuse to other parts of the body before the reaction takes place and hence the second antennæ must have an olfactory function also. Specimens in which both antennules and antennæ were removed showed a marked power of responding to bits of meat or meat juice placed near the mouth parts or the tips of the chelæ. The small chelipeds were found especially sensitive to substances placed near the tip. By very carefully applying the tip of a pipette which had been drawn out into a long slender tube near one of the small chelæ and slowly forcing out a little meat juice, one usually sees a slight grasping movement of the chelæ, often at first a small twitch of the dactyl, followed by movements of reaching about. These movements are followed by exploring movements of the other chelipeds, chewing movements of the mouth parts, and by a turning of the body toward the stimulus.

Animals in which the brain was destroyed were also experimented with. The crayfish was securely fastened to facilitate working upon it. With a fine-pointed scalpel a piece of the carapace about one fourth of an inch square was cut out just over the brain. After destroying the brain the piece of the carapace was carefully replaced and the edges sealed with asphalt varnish. The animal was then kept out of water until the asphalt had dried and the wound sealed. After a few days the animal was experi-

mented with and showed marked responses from the mouth parts and chelipeds. When the chelipeds were rubbed with a piece of filter paper the substance was seized but rejected if passed to the mouth parts. When meat was pressed against the chelipeds in the same way it was seized more eagerly, produced a greater degree of excitement and was generally eaten. To make sure that this was not due to a difference in the consistency of the two articles two wads of filter paper were taken upon one of which was pressed some meat juice. The two wads were applied to the chelipeds in the same way, but the one with the meat juice was more quickly seized and was generally passed to the mouth parts and swallowed. In some instances the pure filter paper was swallowed, but this was very rare.

The chelipeds were also stimulated simultaneously, the one with pure filter paper, the other with filter paper soaked in meat juice. The animal almost invariably turned toward the side with the meat juice and grasped more vigorously on that side. The stimuli were frequently transposed but this did not affect the result; the reaction to the paper with the meat juice was the more vigorous.

In one crayfish the nerve cord was cut across directly behind the mouth parts and in front of the large chelipeds. The result of this operation is to somewhat paralyze the animal, especially the anterior chelipeds which are drawn up under the body. Objects that touch the chelæ are frequently passed to the mouth but again rejected. A bit of meat, however, is chewed and swallowed although this act requires a much longer time than in the normal animal. In some cases the chewing movements ceased before the food was swallowed. The chelipeds when stimulated by meat grasp it more eagerly than they seize innutritious materials, and the animal in general shows a greater degree of excitement.

The experiments recorded show that the outer ramus of the antennules which bear the so-called olfactory setæ are especially sensitive to olfactory stimuli; that the inner ramus of the antennules and the antennæ are also sensitive to olfactory stimuli but to a less degree; and that the olfactory sense is developed in other parts of the body, especially the mouth parts and tips of the chelipeds.